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Practical, enantiospecific syntheses of 14,15-EET and leukotoxin B (vernolic acid)

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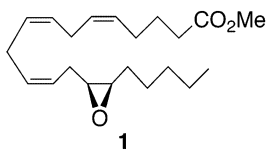
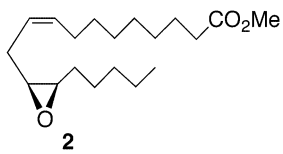
Abstract—Cytochrome P450BM3 and its F87V mutant were exploited for a convenient, laboratory scale (1 mmol) preparation of 14(*S*),15(*R*)-epoxyeicosatrienoic acid [14(*S*),15(*R*)-EET] from arachidonic acid and (+)-leukotoxin B [(+)-12(*S*),13(*R*)-vernolic acid] from linoleic acid, respectively. Their enantiomers were accessed via a four-step chemical inversion.

The cytochrome P450 branch[1] of the eicosanoid cascade has emerged as a physiologically significant source of autacoids. Amongst these, the epoxyeicosatrienoic acids (EETs) and leukotoxins (LTXs), derived from arachidonic acid and linoleic acid, respectively, are amongst the most prominent and widely studied. The EETs induce mitogenesis in epithelial cells,[2] stimulate the release of numerous hormones, biogenic amines, and other cellular mediators,[3] modulate kinases,[4,5] and regulate gene transcription.[6,7] Recent mechanistic studies have linked their mode of action to G_s-proteins[8] and control of ion channels.[9,10] The EETs are also prime candidates for endothelium-derived hyperpolarizing factors (EDHFs) in several species.[11–13] The LTXs are bactericidal/antifungal[14] and have been implicated in a variety of pathophysiologic responses in plants[15] and animals[16,17] including humans.[18,19]

Recent reports of enantiospecific biological responses[20,21] and stereoselective, high affinity binding sites[22] have evoked considerable demand for the individual epoxide antipodes. Since chiral chromatography[23,24] and asymmetric total syntheses[25,26] are expensive and/or tedious, we report herein a convenient, cost effective preparation of both enantiomers of 14,15-EET (**1**) and leukotoxin B (**2**) (LTX-B; vernolic acid). Our strategy exploits the inherently high enzymatic turnover rate and stereoselective metabolism of polyunsaturated fatty acids by the soluble, catalytically self-sufficient cytochrome P450BM3 and its genetically engineered mutants.[27,28] This approach is also applicable to isotopically labeled derivatives using commercial fatty acids.



1



2

For preparative incubations, NADP (2 mL of 50 mM soln in 1% $KHCO_3$), glucose-6-phosphate (10 mL of 200 mM soln in 50 mM MOPS buffer, pH 7.4), and glucose-6-phosphate dehydrogenase (400 μL of 2860 U/mL soln) were added to an oxygen saturated MOPS buffer (3.8 L of 50 mM soln, pH 7.4) forming a NADPH generation and recycling system in a flask open to the atmosphere.[29] After 2 min, arachidonic acid (500 mg in 250 mL deoxygenated 50 mM K_2CO_3) was added followed quickly by 600 units of P450BM3 (F87V).[27] A unit is defined as the amount of enzyme that catalyses the consumption of one micromole of NADPH per minute in the presence of 250 μM arachidonate, 250 μM oxygen, and 250 μM NADPH. After stirring for 15 minutes at room temperature, the incubation was quenched by the dropwise addition of 1 M oxalic acid to pH 4, and extracted with Et_2O (3×250 mL). The combined ethereal extracts were washed with distilled water (3×150 mL), dried over Na_2SO_4, and evaporated to dryness in vacuo. The residue was esterified in ether/methanol (100 mL, 4:1) using excess diazomethane. Silica gel chromatography (10% ethyl

Keywords: eicosanoids; epoxides; inversion reactions; asymmetric synthesis; biosynthesis.

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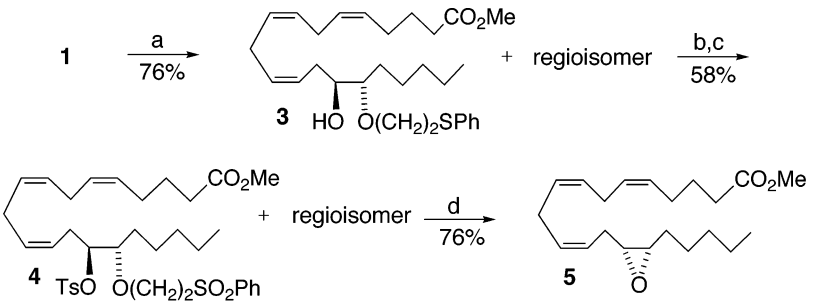

Scheme 1. *Reagents and conditions:* (a) Al_2O_3, $PhS(CH_2)_2OH$ (1.2 equiv.), $(n\text{-}Bu)_2O$, 80°C, 16 h; (b) TsCl, DABCO, CH_2Cl_2, 23°C, 8 h; (c) TPAP (0.1 equiv.), NMO (4 equiv.), CH_3CN, 81°C, 24 h; (d) DBU, CH_3CN, 81°C, 14 h.

acetate/hexane) of the residue left after evaporation of all volatiles afforded methyl 14(*S*),15(*R*)-EET[30] (**1**) (249 mg, 45%) and unreacted methyl arachidonate (260 mg, 50%).

A convenient chemical sequence for the inversion of configuration of an epoxide is outlined in Scheme 1, as illustrated by the transformation of **1** into its antipode. Nucleophilic addition of 2-(phenylthio)ethanol to **1** under the influence of alumina[31] afforded nearly equal amounts of alcohol **3** and its regioisomer.[32] Without separation, this mixture was tosylated and then oxidized using catalytic TPAP, recycled by NMO, to give sulfone **4** and the companion regioisomer. DBU induced β-elimination of the sulfone with concomitant displacement of the tosylate generated methyl 14(*R*),15(*S*)-EET (**5**) in good overall yield.

Comparable enzymatic epoxidation of linoleic acid, preferably with wild type P450BM3,[33,34] and diazomethane esterification provided methyl (+)-leukotoxin B[30] (**2**) [methyl (+)-12(*S*),13(*R*)-vernolate] with a similar efficiency as above. Likewise, repetition of the inversion described in Scheme 1 using **2** gave rise to methyl (−)-leukotoxin B (**6**). Saponification (NaOH, THF/H_2O, 23°C, 6 h; 1N HCl to pH 4.5) of the above esters afforded the corresponding free acids in nearly quantitative yields.

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